

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER GJE-67	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <b>09/856070</b>	
INTERNATIONAL APPLICATION NO. PCT/GB00/03566		INTERNATIONAL FILING DATE 15 September 2000		PRIORITY DATE CLAIMED 17 September 1999	
TITLE OF INVENTION Regulatory/Unfolding Peptides of Ezrin					
APPLICANT(S) FOR DO/EO/US Rupert Donald Holms					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</p> <p>4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p style="margin-left: 20px;">b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> is attached hereto.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> have been communicated by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p style="margin-left: 20px;">d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) _____</p> <p>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p><b>Items 11 to 20 below concern document(s) or information included:</b></p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.</p> <p>14. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</p> <p>15. <input type="checkbox"/> A substitute specification.</p> <p>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 37 CFR 1.821 - 1.825.</p> <p>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</p> <p>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</p> <p>20. <input checked="" type="checkbox"/> Other items or information:</p> <p style="margin-left: 20px;">Entitled to Small Entity Status</p>					

U.S. APPLICATION NO. (if known) <b>09/856070</b>		INTERNATIONAL APPLICATION NO. <b>PCT/GB00/03566</b>		ATTORNEY'S DOCKET NUMBER <b>GJE-67</b>			
21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. .... <b>\$1000.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b>  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>          <table style="width:100%; border: none;"> <tr> <td style="width:50%; border: none;"></td> <td style="width:50%; border: none; text-align: right;">\$860.00</td> </tr> </table>			\$860.00
	\$860.00						
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).							
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$			
Total claims	<u>46</u> - 20 =	<u>26</u>	x <b>\$18.00</b>	\$468.00			
Independent claims	<u>4</u> - 3 =	<u>1</u>	x <b>\$80.00</b>	\$80.00			
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ <b>\$270.00</b>	\$0.00			
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$1.408.00			
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	\$704.00		
<b>SUBTOTAL =</b>				\$704.00			
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$0.00			
<b>TOTAL NATIONAL FEE =</b>				\$704.00			
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +				\$0.00			
<b>TOTAL FEES ENCLOSED =</b>				\$704.00			
				Amount to be refunded:	\$		
				charged:	\$		
a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed. b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>19-0065</u> in the amount of \$ <u>704.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0065</u> . A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. <b>WARNING:</b> Information on this form may become public. <b>Credit card</b> <b>information should not be included on this form.</b> Provide credit card information and authorization on PTO-2038.							
<b>NOTE:</b> Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.							
CORRESPONDENCE ADDRESS:							
CUSTOMER NUMBER <b>23,557</b>			<div style="text-align: center;">          SIGNATURE          David R. Saliwanchik       </div> <div style="text-align: center;">         May 17, 2001          DATE       </div> <div style="text-align: center;">         31,794          REGISTRATION NUMBER       </div>				

I hereby certify that this correspondence is being transmitted to the United States Patent and Trademark Office in an envelope addressed to: Assistant Commissioner for Patents Washington, D.C. 20231 on August 20, 2001

SUBMISSION OF SEQUENCE LISTING AND STATEMENT UNDER 37 CFR §§1.821-1.825 <sup>#3</sup>  
Examining Group (not yet assigned)  
Patent Application  
Docket No. GJE-67  
Serial No. 09/856,070

David Saliwanchik  
David R. Saliwanchik, Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit : (not yet assigned)  
Applicant(s) : Rupert Donald Holms  
Serial No. : 09/856,070  
Filed : May 17, 2001  
For : Regulatory/Unfolding Peptides of Ezrin

Assistant Commissioner for Patents  
Washington, D.C. 20231

SUBMISSION OF SEQUENCE LISTING AND STATEMENT  
UNDER 37 CFR §§1.821-1.825

Sir:

Transmitted herewith in computer readable form and on a paper is a Sequence Listing under 37 CFR §§1.821 through 1.825 for the above-identified patent application. A Notice to Comply with Sequence Rules under 37 CFR §§1.821-1.825 was mailed on June 20, 2001 from the Patent and Trademark Office, and a copy of the Notice is enclosed herewith.

I hereby certify that the attached sequence listing in computer readable form is identical to the attached paper copy of the sequence listing and that no new material is added by this submission.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik  
Patent Attorney  
Registration No. 31,794  
Phone No.: 352-375-8100  
Fax No.: 352-372-5800  
Address: Gainesville, Florida 32606-6669

DRS/srp

Attachments: Sequence Listing on paper; computer readable format containing the same information as paper copy of Sequence Listing; copy of Notice from PTO.

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<120> Regulatory/Unfolding Peptides of Ezrin

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<140> 09/856,070

<141> 2001-05-17

<150> PCT/GB00/03566

<151> 2000-09-15

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<151> 1999-09-17

<160> 28

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PCT09

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PATENT APPLICATION: US/09/856,070

TIME: 10:30:44

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 17 <140> CURRENT APPLICATION NUMBER: 09/856,070  
 19 <141> CURRENT FILING DATE: 2001-05-17  
 23 <150> PRIOR APPLICATION NUMBER: PCT/GB00/03566  
 25 <151> PRIOR FILING DATE: 2000-09-15  
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 31 <151> PRIOR FILING DATE: 1999-09-17  
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PATENT APPLICATION: US/09/856,070

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574 &lt;223&gt; OTHER INFORMATION: Hepreceptor peptide

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581 1 5 10

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## VERIFICATION SUMMARY

PATENT APPLICATION: US/09/856,070

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or

09/856070

J618 Rec'd PCT/PTO 1 7 MAY 2001

May 17, 2001

PRELIMINARY AMENDMENT  
Patent Application

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Rupert Donald Holms  
Docket No. : GJE-67  
For : Regulatory/Unfolding Peptides of Ezrin

Box PCT  
Assistant Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified patent application as follows:

In the Claims

The following amendments are made with respect to the claims in the international application PCT/GB00/03566.

Please cancel claims 1-30 and add the following new claims:

31. A molecule that binds to at least one domain of the Heparin Receptor.
32. The molecule, according to claim 1, which comprises an amino acid sequence which is identical to a portion of the Heparin Receptor.
33. The molecule, according to claim 32, which comprises a sequence of between 5 and 13 amino acids which is identical to a portion of the Heparin Receptor.



34. The molecule, according to claim 32, which comprises an amino acid sequence selected from the group consisting of :

AREEKHQKQLERQQLETEKKRRETVEREKEQM (SEQ ID NO. 1);  
MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ (SEQ ID NO. 2);  
TEKKR (SEQ ID NO. 3);  
TEKKRRETV (SEQ ID NO. 4);  
TEKKRRETVER (SEQ ID NO. 5);  
KKRRE (SEQ ID NO. 6);  
KKRRETVE (SEQ ID NO. 7);  
KKRRETVERE (SEQ ID NO. 8);  
KKRRETVEREK (SEQ ID NO. 9);  
KKRRETVEREKE (SEQ ID NO. 10);  
KRRETVER (SEQ ID NO. 11);  
KRRETVEREK (SEQ ID NO. 12);  
KRRETVEREKE (SEQ ID NO. 13);  
RRETV (SEQ ID NO. 14);  
RETVEREKE (SEQ ID NO. 15);  
EREKE (SEQ ID NO. 16);  
EREKEQMMREKEEL (SEQ ID NO. 17);  
KEELM (SEQ ID NO. 18);  
KEELMLRLQDYEE (SEQ ID NO. 19);  
KEELMLRLQDY<sub>p</sub>EE (SEQ ID NO. 20);  
EELMLRLQDYEE (SEQ ID NO. 21);  
EELMLRLQDY<sub>p</sub>EE (SEQ ID NO. 22);  
ELMLRLQDYEE (SEQ ID NO. 23);  
ELMLRLQDY<sub>p</sub>EE (SEQ ID NO. 24);  
MLRLQ (SEQ ID NO. 25);

QDYEE (SEQ ID NO. 26); and

QDYpEE (SEQ ID NO. 27).

35. The molecule, according to claim 34, which comprises:

AREEKHQKQLERQQLETEKKRRETVEREKEQM (SEQ ID NO. 1).

36. The molecule, according to claim 34, which comprises:

MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ (SEQ ID NO. 2).

37. The molecule, according to claim 34, which comprises:

TEKKR (SEQ ID NO. 3).

38. The molecule, according to claim 34, which comprises:

TEKKRRETV (SEQ ID NO. 4).

39. The molecule, according to claim 34, which comprises:

TEKKRRETVER (SEQ ID NO. 5).

40. The molecule, according to claim 34, which comprises:

KKRRE (SEQ ID NO. 6).

41. The molecule, according to claim 34, which comprises:

KKRRETVE (SEQ ID NO. 7).

42. The molecule, according to claim 34, which comprises:

KKRRETVERE (SEQ ID NO. 8).

43. The molecule, according to claim 34, which comprises:  
KKRRETVEREK (SEQ ID NO. 9).

44. The molecule, according to claim 34, which comprises:  
KKRRETVEREKE (SEQ ID NO. 10).

45. The molecule, according to claim 34, which comprises:  
KRRETVER (SEQ ID NO. 11).

46. The molecule, according to claim 34, which comprises:  
KRRETVEREK (SEQ ID NO. 12).

47. The molecule, according to claim 34, which comprises:  
KRRETVEREKE (SEQ ID NO. 13).

48. The molecule, according to claim 34, which comprises:  
RRETV (SEQ ID NO. 14).

49. The molecule, according to claim 34, which comprises:  
RETVEREKE (SEQ ID NO. 15).

50. The molecule, according to claim 34, which comprises:  
EREKE (SEQ ID NO. 16).

51. The molecule, according to claim 34, which comprises:  
EREKEQMMREKEEL (SEQ ID NO. 17).

52. The molecule, according to claim 34, which comprises:  
KEELM (SEQ ID NO. 18).

53. The molecule, according to claim 34, which comprises:  
KEELMLRLQDYEE (SEQ ID NO. 19).

54. The molecule, according to claim 34, which comprises:  
KEELMLRLQDYpEE (SEQ ID NO. 20).

55. The molecule, according to claim 34, which comprises:  
EELMLRLQDYEE (SEQ ID NO. 21).

56. The molecule, according to claim 34, which comprises:  
EELMLRLQDYpEE (SEQ ID NO. 22).

57. The molecule, according to claim 34, which comprises:  
ELMLRLQDYEE (SEQ ID NO. 23).

58. The molecule, according to claim 34, which comprises:  
ELMLRLQDYpEE (SEQ ID NO. 24).

59. The molecule, according to claim 34, which comprises:  
MLRLQ (SEQ ID NO. 25).

60. The molecule, according to claim 34, which comprises:  
QDYEE (SEQ ID NO. 26).

61. The molecule, according to claim 34, which comprises:

QDYpEE (SEQ ID NO. 27).

62. A method for upregulating the immune system wherein said method comprises administering, to a patient in need of such upregulation, an effective amount of a molecule which binds to the Heparin receptor.

63. The method, according to claim 62, wherein said molecule is charged.

64. The method, according to claim 62, wherein said molecule comprises an amino acid sequence identical to all or part of the Heparin receptor.

65. The method, according to claim 64, wherein said molecule comprises between 5 and 13 amino acids which are identical to the Heparin receptor.

66. The method, according to claim 64, wherein said molecule comprises an amino acid sequence selected from the group consisting of :

AREEKHQKQLERQQLETEKKRRETVEREKEQM (SEQ ID NO. 1);

MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ (SEQ ID NO. 2);

TEKKR (SEQ ID NO. 3);

TEKKRRETV (SEQ ID NO. 4);

TEKKRRETVER (SEQ ID NO. 5);

KKRRE (SEQ ID NO. 6);

KKRRETVE (SEQ ID NO. 7);

KKRRETVERE (SEQ ID NO. 8);

KKRRETVEREK (SEQ ID NO. 9);

KKRRETVEREKE (SEQ ID NO. 10);

KRRETVER (SEQ ID NO. 11);  
KRRETVEREK (SEQ ID NO. 12);  
KRRETVEREKE (SEQ ID NO. 13);  
RRETV (SEQ ID NO. 14);  
RETVEREKE (SEQ ID NO. 15);  
EREKE (SEQ ID NO. 16);  
EREKEQMMREKEEL (SEQ ID NO. 17);  
KEELM (SEQ ID NO. 18);  
KEELMLRLQDYEE (SEQ ID NO. 19);  
KEELMLRLQDYpEE (SEQ ID NO. 20);  
EELMLRLQDYEE (SEQ ID NO. 21);  
EELMLRLQDYpEE (SEQ ID NO. 22);  
ELMLRLQDYEE (SEQ ID NO. 23);  
ELMLRLQDYpEE (SEQ ID NO. 24);  
MLRLQ (SEQ ID NO. 25);  
QDYEE (SEQ ID NO. 26); and  
QDYpEE (SEQ ID NO. 27).

67. A method for treating tumors wherein said method comprises administering, to a patient in need of such treatment, an effective amount of a molecule which binds to at least one domain of the Heparin Receptor.

68. The method, according to claim 67, wherein said molecule is charged.

69. The method, according to claim 67, wherein said molecule comprises an amino acid sequence identical to all or part of the Heparin Receptor.

70. The method, according to claim 69, wherein said molecule comprises between 5 and 13 amino acids which are identical to the Hepreceptor.

71. The method, according to claim 67, wherein said molecule comprises an amino acid sequence selected from the group consisting of :

AREEKHQKQLERQQLETEKKRRETVEREKEQM (SEQ ID NO. 1);

MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ (SEQ ID NO. 2);

TEKKR (SEQ ID NO. 3);

TEKKRRETV (SEQ ID NO. 4);

TEKKRRETVER (SEQ ID NO. 5);

KKRRE (SEQ ID NO. 6);

KKRRETVE (SEQ ID NO. 7);

KKRRETVERE (SEQ ID NO. 8);

KKRRETVEREK (SEQ ID NO. 9);

KKRRETVEREKE (SEQ ID NO. 10);

KRRETVER (SEQ ID NO. 11);

KRRETVEREK (SEQ ID NO. 12);

KRRETVEREKE (SEQ ID NO. 13);

RRETV (SEQ ID NO. 14);

RETVEREKE (SEQ ID NO. 15);

EREKE (SEQ ID NO. 16);

EREKEQMMREKEEL (SEQ ID NO. 17);

KEELM (SEQ ID NO. 18);

KEELMLRLQDYEE (SEQ ID NO. 19);

KEELMLRLQDY<sub>p</sub>EE (SEQ ID NO. 20);

EELMLRLQDYEE (SEQ ID NO. 21);

EELMLRLQDY<sub>p</sub>EE (SEQ ID NO. 22);

ELMLRLQDYEE (SEQ ID NO. 23);  
ELMLRLQDYpEE (SEQ ID NO. 24);  
MLRLQ (SEQ ID NO. 25);  
QDYEE (SEQ ID NO. 26);  
QDYpEE (SEQ ID NO. 27); and  
TEKKRRETVEREKE (SEQ ID NO. 28).

72. A method for treating HIV wherein said method comprises administering, to a patient in need of such treatment, an effective amount of a molecule which binds to at least one domain of the Hepreceptor, and wherein said molecule is not Hep 1.

73. The method, according to claim 72, wherein said molecule is charged.

74. The method, according to claim 72, wherein said molecule comprises an amino acid sequence identical to all or part of the Hepreceptor.

75. The method, according to claim 74, wherein said molecule comprises between 5 and 13 amino acids which are identical to the Hepreceptor.

76. The method, according to claim 72, wherein said molecule comprises an amino acid sequence selected from the group consisting of :

AREEKHQKQLERQQLETEKKRRETVEREKEQM (SEQ ID NO. 1);  
MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ (SEQ ID NO. 2);  
TEKKR (SEQ ID NO. 3);  
TEKKRRETV (SEQ ID NO. 4);  
TEKKRRETVER (SEQ ID NO. 5);  
KKRRE (SEQ ID NO. 6);



KKRRETVE (SEQ ID NO. 7);  
KKRRETVERE (SEQ ID NO. 8);  
KKRRETVEREK (SEQ ID NO. 9);  
KKRRETVEREKE (SEQ ID NO. 10);  
KRRETVER (SEQ ID NO. 11);  
KRRETVEREK (SEQ ID NO. 12);  
KRRETVEREKE (SEQ ID NO. 13);  
RRETV (SEQ ID NO. 14);  
RETVEREKE (SEQ ID NO. 15);  
EREKE (SEQ ID NO. 16);  
EREKEQMMREKEEL (SEQ ID NO. 17);  
KEELM (SEQ ID NO. 18);  
KEELMLRLQDYEE (SEQ ID NO. 19);  
KEELMLRLQDYpEE (SEQ ID NO. 20);  
EELMLRLQDYEE (SEQ ID NO. 21);  
EELMLRLQDYpEE (SEQ ID NO. 22);  
ELMLRLQDYEE (SEQ ID NO. 23);  
ELMLRLQDYpEE (SEQ ID NO. 24);  
MLRLQ (SEQ ID NO. 25);  
QDYEE (SEQ ID NO. 26); and  
QDYpEE (SEQ ID NO. 27).

FILED "04092000"

Remarks

Claims 1-30 have been canceled and new claims 31-76 have been added.

No new matter has been added by these amendments.

The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Respectfully submitted,



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Gainesville, FL 32606

DRS/la

10/25/07 04:53:00

**Regulatory/Unfolding Peptides of Ezrin**  
**BACKGROUND TO THE INVENTION**

The field of the present invention relates to the treatment of infectious disease and cancer by inducing disease fighting immune responses. The growing problem of new strains of pathogenic bacteria resistant to antibiotics, the limited range of compounds effective against chronic viral and fungal infections and shortage of effective anti-cancer treatments demonstrates the need for compounds that can enhance the host defence against these medical problems. This invention relates to novel charged molecules which stimulate immune responses by binding to the Heptreceptor, a novel active site in human ezrin which I have discovered. The preferred charged molecules are novel peptides with sequences identical to the Heptreceptor in human ezrin.

Ezrin is a member of the ERM (ezrin-radixin-moesin) family of proteins which play structural and regulatory roles in a wide range of cell types. There is considerable evidence to indicate that ezrin regulates the structure of the cortical cytoskeleton to control cell surface topography. Ezrin adopts two main conformations: 1) a soluble folded form which is found in the cytoplasm and, 2) an unfolded and elongated form which is found attached to the cytoplasmic surface of the cell membrane particularly in conjunction with microvilli and other activation related structures. The N terminal domain of the protein is attached to the cytoplasmic surface of the membrane while the C terminal part binds to the actin cytoskeleton. Ezrin is a tyrosine kinase substrate in T cells and is also tyrosine phosphorylated as a result of Epidermal Growth Factor (EGF) stimulation of the EGF receptor. The N terminal domain of ezrin in its extended conformation binds to the cytoplasmic tail of CD44 in the presence of PIP<sub>2</sub>. Ezrin also may bind to the cytoplasmic tail of ICAM-2. Ezrin is very sensitive to regulatory proteases such as calpain and is rapidly turned over during cell activation.

Anthony Bretscher, David Reczek and Mark Berryman (1997)

"Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures" *Journal of Cell Science* 110: 3011-3018

Detailed analysis of the secondary structure of ezrin shows that there are three main structural domains: an N terminal domain from amino acids 1 to 300, a highly charged alpha domain from amino acids 300 to 470 and C terminal domain from amino acids 470 to 585. Structural modelling suggests that the alpha domain is folded into two anti-parallel helices in the soluble globular form of ezrin although the location of the hinge has not been identified. In the model of the extended phosphorylated form, ezrin is attached to the inner surface of the cell membrane by the N terminal domain, the alpha domains of two ezrin molecules are paired into anti-parallel dimers and located below the cell surface membrane. In this extended form, ezrin is tyrosine phosphorylated at tyrosine 353 (Yp 353).

Ossi Turunen, Markku Sainio, Juha Jaaskelainen, Olli Carpen, Antti Vaheri (1998) "Structure - Function relationships in the ezrin family and the effect of tumor-associated point mutations in neurofibromatosis 2 protein" *Biochimica et Biophysica Acta* 1387: 1-16

I disclosed in United States Patent 5,773,573 that the fourteen amino acid peptide HEP1, (amino acid sequence of TEKRRETEREKE, SEQ ID 28, identical to amino acids 324-337 of human ezrin) which has a 70% identity to the C terminus of gp120 could inhibit HIV replication in vivo in man. At the time I believed that the observed anti-HIV effect of peptide HEP1 was due to the orally administered HEP1 inducing

**TABLE 1 Amino acids, three letter code, one letter code and side chain charges**

CHARGES ON AMINO ACID SIDE CHAINS AT PHYSIOLOGICAL pH				
Amino acid	Three letter code	One letter code	Charge	Symbol
Glycine	Gly	G	NONE	
Alanine	Ala	A	NONE	
Valine	Val	V	NONE	
Isoleucine	Ile	I	NONE	
Leucine	Leu	L	NONE	
Serine	Ser	S	NONE	
Threonine	Thr	T	NONE	
Aspartic acid	Asp	D	NEGATIVE	--
Glutamic acid	Glu	E	NEGATIVE	--
Phosphotyrosine	Tyr(P)	Yp	NEGATIVE	--
Asparagine	Asn	N	WEAK NEGATIVE	-
Glutamine	Gln	Q	WEAK NEGATIVE	-
Lysine	Lys	K	POSITIVE	+
Arginine	Arg	R	POSITIVE	+
Histidine	His	H	WEAK POSITIVE	+
Proline	Pro	P	NONE	
Tryptophan	Trp	W	NONE	
Phenylalanine	Phe	F	NONE	
Tyrosine	Tyr	Y	NONE	
Methionine	Met	M	NONE	
Cysteine	Cys	C	NONE	

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1 is a diagram of the alignments of the primary amino acid sequences of:

- a) the folded anti-parallel associated helices of the Heparceptor in soluble ezrin.
- b) the unfolded helix of the Heparceptor in membrane associated ezrin with an example of a peptide ligand.
- c) two unfolded Heparceptors forming a dimer of anti-parallel associated helices during an interaction between two cells.

FIGURE 2 is an illustration of the relationship between the Heparceptor on ezrin, its ligands, cell membranes, cell surface receptors and cytoskeletal components.

**BRIEF DESCRIPTION OF THE SEQUENCES**

SEQ ID 1 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 2 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 3 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 4 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 5 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 6 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 7 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 8 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 9 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 10 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 11 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 12 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 13 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 14 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 15 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 16 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 17 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 18 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 19 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 20 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 21 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 22 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 23 is an amino acid sequence of a peptide according to the present invention.

SEQ ID 24 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 25 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 26 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 27 is an amino acid sequence of a peptide according to the present invention.  
SEQ ID 28 is an amino acid sequence of a peptide according to the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

The soluble conformation of ezrin found in the cytoplasm comprises of two adjacent alpha helical domains which are folded together at a hinge region (M339-M340) into two anti-parallel helices stabilised by complimentary side chain charges of the primary amino acid sequence. It is the subject of this invention that the positively and negatively charged side chains of the amino acid sequence of Heptreceptor Domain A are complementary to the positively and negatively charged side chains of the amino acid sequence of the Heptreceptor Domain B. In the activated open conformation of ezrin, the interaction of the Domain A and Domain B of the Heptreceptors of two different ezrin molecules allows the formation of anti-parallel dimers. In addition to the antiparallel dimers of ezrin which form below the cell surface, I have determined that these dimers can form between a Heptreceptor exposed on the surface on one cell with a Heptreceptor exposed on the surface of another cell. When the two Heptreceptors make contact during close association of two cell surfaces an activation signal is initiated in both cells (FIGURE 2). Any charged molecule that partially mimics the interaction between the side chains charges of Domain A and Domain B of the Heptreceptors will give rise to a medically useful biological response.

Heptreceptor-Domain A (amino acid numbers 308-339 of human ezrin), comprises of the following 32 amino acid sequence.

(The sequences are listed using the single letter code for each amino acid written from the N terminus to the C terminus of the polypeptide. Yp represents the form of phosphotyrosine found in vivo)

SEQ ID 1

AREEKHQKQLERQQLETEKKRRRETVEREKEQM

In United States Patent 5,773,573, I disclosed the anti-HIV activity of peptide HEP1 (SEQ ID 28) which I have now discovered has a sequence identical to part of Heptreceptor-Domain A (spanning amino acids 324-337 of the human ezrin sequence). In the above patent I made the assumption that anti-HIV activity was due to the induction of immunological tolerance to autoreactive immune responses induced by the C terminus of HIV gp120. I can now disclose that the anti-HIV activity of HEP1 is due to its binding to Heptreceptor Domain B and the induction of a novel immune response. It is a subject of this invention that there are novel peptides derived from the Heptreceptor of ezrin with significantly superior activity to HEP1.

Heptreceptor-Domain B (amino acid numbers 340-373 of human ezrin), comprises of the following 34 amino acid sequence (Tyrosine 353 [Y] may be phosphorylated to phosphotyrosine [Yp] in the membrane associated conformation of ezrin):

SEQ ID 2

MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEIQRALQ

I have determined that Domain B of the Heptreceptor is the site on ezrin to which HIV gp120 binds during infection of the brain. (HIV gp120 binds to Heptreceptor Domain B using its charged C terminal amino acids which have a 70% identity to part of Heptreceptor Domain A). Novel charged molecules which bind to the Heptreceptor may be useful in treating HIV related dementia.

Claudia Hecker, Christoph Weise, Jurgen Schneider-Schaulies, Harvey Holmes, Volker ter Meulen (1997) "Specific binding of HIV-1 envelope protein gp120 to the structural membrane proteins ezrin and moesin." Virus Research 49 215-223



I have demonstrated (EXAMPLE 1A) that Hpreceptor peptides have significant adjuvant activity and this is demonstrated by enhancing the IgG antibody response to Ovalbumin in mice using HEP1, Rupe312, Rupe1014, Rupe1024 and Rupe2032. I have also demonstrated (EXAMPLE 1B) the activity of Hpreceptor peptides in enhancing the antibody-dependent cytotoxic response in Thymus to Sheep Red Blood Cells (SRBC) in mice using HEP1, Rupe312, Rupe15, Rupe1014, Rupe1024 and Rupe2032.

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe15 SEQ ID 3: TEKKR and Rupe1014 SEQ ID 16: EREKE and Rupe1024 SEQ ID 17: EREKEQMMREKEEL and Rupe2032 SEQ ID 19: KEELMLRLQDYEE and HEP1 SEQ ID 28: TEKKRRETVEREKE)

I have demonstrated (EXAMPLE 2) that hepreceptor peptides have significant anti-tumour activity and this is demonstrated by Hpreceptor peptides reducing the growth rate of fast growing transplanted sarcomas and slower growing transplanted cervical cancer in mice using Rupe312 and Rupe414.

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13: KRRETVEREKE)

I have demonstrated (EXAMPLE 3) that HEP1 therapy (10mg per day orally either for thirty days or ninety days) in 21 HIV infected patients induces immune responses which leads to clinical improvement over the following six months after therapy, as measured by an increasing CD4 T lymphocyte population and declining opportunistic infections, declining HIV infectivity and declining CD38,CD8 population of T lymphocytes (an established prognostic marker of the progression to AIDS).

M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)

"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* 90 376-382

A mean increase in the level of expression of CD44 and MHC Class I on T lymphocytes over six months was observed which appears to also correlate with the clinical improvement. No toxicity was detected with the administration of HEP1. Increases of MHC Class I expression and CD44 expression are associated with increases in memory T cells and Class I restricted cell mediated immunity.

Stephan Oehen and Karin Brduscha-Riem (1998)

"Differentiation of Naïve CTL to Effector and Memory CTL: Correlation of Effector Function with Phenotype and Cell Division" *The Journal of Immunology* 161 5338-5346

The results of this trial demonstrates that a peptide or other charged molecule which mimics all or part of the Hpreceptor can give rise to an activation signal that eventually leads to a change in the homeostasis of the immune system and long term up regulation of cell mediated and humoral immunity. I have also demonstrated that acute and chronic candida infection in women can be treated and cured by the immune response arising from Hpreceptor stimulation (EXAMPLE 4). I have demonstrated that peptides derived from the Hpreceptor can activate monocytes and macrophages in mice both in vitro and in vivo, which leads to a protective immune response. (EXAMPLE 5). Peptides of this invention have a significantly higher activity than HEP1. Hpreceptor stimulation also leads to the activation of human peripheral blood mononuclear cells which was demonstrated by measuring the incorporation of radioactive tritiated thymidine into DNA of the growing cells. Novel peptides, Rupe312 and Rupe414 derived from Hpreceptor Domain A had a ten fold higher activity than HEP1. (EXAMPLE 6)

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13: KRRETVEREKE)

I also discovered that a 24 hour incubation of human White Blood Corpuscles (WBC) with peptides derived from the Hpreceptor results in a fall in MHC Class I cell surface expression, probably due to cell activation and receptor internalisation, and an increase in the total population of macrophages expressing MHC Class I. This is consistent with the long term increase in the population cells expressing MHC Class I seen in HIV patients during the six months following HEP1 therapy. In this assay system Rupe312 and Rupe414 had significantly higher activity than HEP1 (EXAMPLE 7).

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13: KRRETVEREKE)

I have demonstrated (EXAMPLE 8) that hpreceptor peptides have significant suppressive effect on the expression of IL-8. The inhibition of IL-8 may play a role in the selective activity of hpreceptor peptides in activating monocytes/macrophages (Rupe312 SEQ ID 8:KKRRETVERE)

I have demonstrated (EXAMPLE 9) that very low dose Hpreceptor peptides (1-100 nanograms/mouse) protect mice from acute infection by Salmonella tryphimurium (Rupe15 SEQ ID 3: TEKKR and Rupe1024 SEQ ID 17: EREKEQMMREKEEL and HEP1 SEQ ID 28: TEKKRRETVEREKE)

I have demonstrated (EXAMPLE 10) that very low dose Hpreceptor peptides (1-1000 nanograms/mouse) enhance survival time in a mouse lethal herpes virus infection model. (HEP1 SEQ ID 28: TEKKRRETVEREKE)

This invention describes charged molecules which specifically bind to the Hpreceptor. I have designed three groups of novel charged peptides which have sequences identical to the amino acid sequences of the complementary domains of the Hpreceptor and which either bind to Hpreceptor Domain B (SEQ ID 3 - SEQ ID 16), or to both Domain A and B (SEQ ID 17), or which bind to Domain A (SEQ ID 18 - SEQ ID 27). The peptides which are a subject of this invention probably bind to cell surface exposed Hpreceptors and stabilise the unfolded conformation of ezrin and induce immuno-modulatory effects. The preferred peptides are between five and thirteen amino acids in length and the preferred sequences are as follows.

Hpreceptor Domain B binding peptides:

SEQ ID 3	
Rupe15:	TEKKR
SEQ ID 4	
Rupe19:	TEKKRRETV
SEQ ID 5	
Rupe111:	TEKKRRETVER
SEQ ID 6	
Rupe37:	KKRRE
SEQ ID 7	
Rupe310:	KKRRETVE

SEQ ID 8  
Rupe312: KKRRETVERE

SEQ ID 9  
Rupe313: KKRRETVEREK

SEQ ID 10  
Rupe314: KKRRETVEREKE

SEQ ID 11  
Rupe411: KRRETVER

SEQ ID 12  
Rupe413: KRRETVEREK

SEQ ID 13  
Rupe414: KRRETVEREKE

SEQ ID 14  
Rupe59: RRETV

SEQ ID 15  
Rupe614: RETVEREKE

SEQ ID 16  
Rupe1014: EREKE

## Hepreceptor Domain A and Domain B binding peptide:

SEQ ID 17  
Rupe1024 EREKEQMMREKEEL

## Hepreceptor Domain A binding peptide:

SEQ ID 18  
Rupe2024: KEELM

SEQ ID 19  
Rupe2032: KEELMLRLQDYEE

SEQ ID 20  
Rupe2032p: KEELMLRLQDYpEE

SEQ ID 21  
Rupe2132: EELMLRLQDYEE

SEQ ID 22  
Rupe2132p: EELMLRLQDYpEE

SEQ ID 23  
Rupe2232: ELMLRLQDYEE

SEQ ID 24  
Rupe2232p: ELMLRLQDYpEE

SEQ ID 25  
Rupe2428: MLRLQ

SEQ ID 26  
Rupe2832: QDYEE

SEQ ID 27  
Rupe2832p: QDYpEE

Other peptides or other charged molecules which bind to Domain A or Domain B or bridge Domain A and Domain B of the Hepreceptor are likely to be biologically active. These peptides or other charged molecules can be administered orally and by other routes for the treatment of various infectious diseases and cancer.

## HOW TO MAKE

Peptides used in this invention may be synthesised for example, using a solid phase method using either Boc or Fmoc chemistry or any other practical route for peptide synthesis known to those skilled in the art of peptide synthesis.

Stepwise solid phase synthesis with Boc-amino acids can be performed based on the method of Merrifield; (Journal of American Chemical Society 85 2149-2154). The following compounds can be used (Novabiochem resin: Boc-Glu(OBzl)-PAM, Amino acids: Boc-Lys (2Cl-Z)-OH, Boc-Glu(OBzl)OH, Boc-Arg(Tos)OH, Boc-Val-OH, Boc-Thr (Bzl)-OH, Solvents: DMF (Rathburn), Dichloromethane (BDH), Ethylacetate (BDH), Reagents: HBTU (Phase Separations Ltd), p-Cresol (Lanchester) TFA (Halocarbon Products Corporation) HF (BOC) DIEA (Fluka). Recommended reactive side chain protecting groups for Boc-amino acids are: Arg (Tos), Asn (Xan), Asp (OcHxl), Glu (OcHxl) Gln (Xan) or Gln, His (DNP), Lys (CIZ), Serine (Bzl) Tyr (BrZ) Trp (CHO). The abbreviations have the following meanings: DCC=Dicyclohexylcarbodiimide, DIC=Diisopropylcarbodiimide, DCM=Dichloromethane, DMF=Dimethylformamide, TFA=Trifluoroacetic acid, Boc= t-Butyloxycarbonyl, HOBT=Hydroxybenzotriazole, DIEA=Diisopropylethylamine, DCU=Dicyclohexylurea.

For example, boc synthesis of a peptide of this invention could be performed as follows: HBTU activation/in situ neutralization on 0.5 mmol scale uses 0.5 mmol of resin and a three fold excess of activated Boc amino acid. Boc amino acid and activating reagent (HBTU) should be used in equimolar quantities ie 2mmol each in this case equals a 3x excess. DIEA is used to both neutralise the resin for coupling and to activate the Boc-amino acid. (Hence 2.5 mmol is used, 1 equivalent Boc-aa and 1 equivalent resin). Reagents: 0.5M HBTU in DMF (MW=379, 0.5M=18.95g in 100ml, note it is not light stable) requires 2mmol=4ml and 2.5 mmol DIEA=0.46ml (MW=129, d=0.742). Activation of aminoacids: Boc-amino acid should be activated only immediately prior to addition to the resin, especially in the case of Arg (Tos). For all Boc amino acids: weigh 2 mmol Boc amino acid into a 20 ml glass sample bottle. Add 4ml 0.5M HBTU solution and shake to dissolve solid. Add 0.46 ml DIEA and mix (some colour change may be observed). Method: wash resin with DMF, remove Boc-protecting group with 100% TFA- Shake twice for 1 minute draining in between, drain, flow wash with DMF for 1 minute, drain, add activated amino acid solution, shake for 10 minutes, then take sample and perform the ninhydrin test to determine coupling efficiency. On completion of the synthesis flow wash with DMF, then DCM and dry. The synthesis of the first and every subsequent level of peptide construction is achieved using a three fold excess of HBTU activated Boc-amino acids in DMF. In all couplings, the coupling efficiency should be more than 99% as indicated by quantitative ninhydrin testing. Deprotection of the N-termini is performed in 100% TFA. The resin peptide is carefully flow-washed before and after the deprotection. After the last coupling and removal of the Boc-protection, the peptide resin is washed with dichloromethane and dried by air. The peptide is removed from the resin support by the high HF method (2 g resin peptide, 2g cresol, 20ml HF, 1.5 h

at  $-5^{\circ}\text{C}$ ) to yield the crude peptide which is precipitated with ethylacetate (100ml) and redissolved in 6M guanidine HCL-0.1M TRIS solution (20ml).

The peptide can be purified as follows using an analytical HPLC separation on a Vydac  $\text{C}_{18}$  5 RAC column. HPLC grade acetonitrile (aldrich) and water is filtered through a membrane filter and degassed with helium flow prior to use. Analytical separation achieved with a solvent gradient beginning with 0% acetonitrile, increasing constantly to 60% acetonitrile at 20 minutes, staying at this concentration for twenty minutes and decreasing steadily to 0% acetonitrile for 10 minutes at a constant flow of 1.2 ml per minute. Preparative separation of peptide achieved on a TSK-GEL preparative  $\text{C}_{18}$  column. Separation is achieved with a solvent gradient beginning with 0% acetonitrile, increasing constantly to 18% acetonitrile at 60 minutes, then 60% acetonitrile for 80 minutes, staying at this concentration for 30 minutes at a constant flow of 8 ml per minute. The gradient can be controlled by two microprocessor controlled Gilson 302 single piston pumps. Compounds can be detected with a Waters 486 Tunable Absorbance Detector at 214 nm and analytical chromatographs recorded with an HP laserjet 5L. A Holochrome UV-VIS detector 220 nm for preparative chromatographs can be recorded with an LKB 2210 single channel recorder. Capillary Electrophoresis quality control can be carried out using Waters Quanta 4000 equipment using a phosphate buffer (75 microM) pH2.5 run at 15 kV, sample time 20 seconds, loaded by hydrostatic injection on 60 cm column, run time 12 minutes. The yield for 1 g 0.46 mmol resin synthesis should be about 250 mg pure peptide

Alternative solution synthesis methods may also be used to produce larger quantities of the peptides of this invention. Protected trimer fragments can be obtained using stepwise synthesis by the active esters method known to those skilled in the art of peptide synthesis. The fragments are then assembled using DCC/HOBT after removal of relevant C and N terminal protective groups. After removal of all protective groups the crude peptide is partially purified on SP-Sephadex-C25 ion exchange chromatography followed by preparative HPLC then lyophilised.

#### HOW TO USE

0.01 to 1000 mg of lyophilised peptide may be dissolved in 1-10 ml distilled water and administered orally or vaginally. 0.01 to 1000 mg may be formulated in to a pill or capsule or suppository with carriers used commonly by those skilled in the art of pill or capsule or suppository manufacture and administered orally or vaginally or anally. A filter sterilized solution of between 0.001 and 100 milligrams of peptide in distilled water may be injected subcutaneously or intravenously or intramuscularly.

The following Examples serve to illustrate the invention only, and should not be construed as limiting it in any way.

**EXAMPLE 1A****Adjuvant activity of Hепreceptor peptides enhancing the IgG serum antibody response to Ovalbumin in mice.**

The IgG response in mice two weeks after an injection of 50 micrograms of Ovalbumin plus various concentrations of different hepreceptor peptides was determined. IgG response was measured by Optical Density (OD) of a 1 to 100 dilution of mouse sera in the presence of Ovalbumin.

**Results**

IgG response recorded as OD

Peptide code	Control	IP injection in micrograms per mouse				Peptide sequence
	0	0.01	0.1	1	10	
HEP1	0.69	0.45	0.44	0.56	0.57	TEKKRRETVEREKE
Rupe312	0.69	0.68	0.88	0.55	0.44	KKRRETVERE
Rupe1014	0.127	0.232	0.231	0.477	0.508	EREKE
Rupe1024	0.152	0.250	0.262	0.489	0.445	EREKEQMMREKEEL
Rupe2032	0.127	0.509	0.606	0.327	0.203	KEELMLRLQDYEE

IgG response recorded as OD then data rebased relative to 100 for each control

Peptide code	Control	IP injection in micrograms per mouse				Peptide sequence
	0	0.01	0.1	1	10	
HEP1	100	65	64	81	83	TEKKRRETVEREK
Rupe312	100	99	128	80	64	KKRRETVERE
Rupe1014	100	183	182	376	400	EREKE
Rupe1024	100	164	172	322	293	EREKEQMMREKEEL
Rupe2032	100	401	477	257	160	KEELMLRLQDYEE

**Conclusion**

All the hepreceptor peptides with the exception of HEP1 show adjuvant activity but with different optimal concentrations.

**EXAMPLE 1B****Activity of Hепreceptor peptides enhancing the antibody-dependent cytotoxic response in Thymus to Sheep Red Blood Cells (SRBC) in mice.**

The influence of Hепreceptor peptides on the activation of antibody forming cells against sheep erythrocytes (SRBC) in Mice (CBA - C57Bl Fi hybrids, 2months old, weight 18-22 grams) was determined. Mice were first injected intraperitoneally using either 0.5 ml sterile saline as a control or Hепreceptor peptides in the same volume of saline. 30 minutes after the injection of Hепreceptor peptides, a cell suspension containing 5 million SRBC was administered intraperitoneally to each mouse. Four days after the immunisation, the mice were killed by cervical dislocation and the spleens were obtained aseptically. Each spleen was homogenised in 2ml of Medium 199, then 100 microlitres of this suspension was put into 1ml of prepared agarose in

Medium 199 (stored in water bath at 48°C), SRBC suspension was added, resulting in a final concentration of 1% agarose. The 1ml of agarose-cell mixture was agitated then transferred to petri dish to set. When the agarose became solid, the petri dish was incubated for 1 hour at 37°C then 0.5 ml of 1-in-20 diluted guinea pig serum in Medium 199 was added on top of agarose gel as a source of complement. The incubation was continued for another 1 hour. The dishes were then visualised using an 8x binocular microscope and the number of plaques counted ( each plaque equivalent to one antibody secreting mouse spleen cell). The results were expressed both as the number of antibody secreting cells per one million nucleated spleen cells and the number of antibody secreting cells per whole spleen. As no mitogenic effect was observed (Hepreceptor peptide administration resulted in normal sized mouse spleens), these two calculations gave similar results.

Averaged data (from thirty mice per data point) of the number of antibody forming cells per one million nucleated spleen cells. The data was re-based relative to 100 for each control group.

Peptide code	Control	IP injection in micrograms per mouse		Peptide sequence
		0	10	
HEP1	100	192	203	TEKKRRETVEREKE
Rupe312	100	317	267	KKRRETVERE
Rupe15	100	255	134	TEKKR
Rupe1014	100	202	325	EREKE
Rupe1024	100	401	397	EREKEQMMREKEEL
Rupe2032	100	236	232	KEELMLRLQDYEE

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe15 SEQ ID 3: TEKKR and Rupe1014 SEQ ID 16: EREKE and Rupe1024 SEQ ID 17: EREKEQMMREKEEL and Rupe2032 SEQ ID 19: KEELMLRLQDYEE and HEP1 SEQ ID 28: TEKKRRETVEREKE)

### Conclusion

All the Hepreceptor peptides enhance the antibody-dependent cytotoxic response in thymus but with different optimal concentrations.

### EXAMPLE 2

**Anticancer activity of hepreceptor peptides on the growth of transplanted sarcoma of the uterus SM-322 and on cancer of the uterine cervix CUS-5 performed in CBA mice using Rupe312 and Rupe414 as examples.**

### Materials and Methods

#### *Transplanted mouse sarcoma SM-322 model (endothelial tumour)*

Primary tumours of the uterus were induced in female mice using 1,2 dimethylhydrazine. A few primary tumours were transplanted into syngeneic mice. The first visible nodules started to appear at the point of transplation after 4 days. The life expectancy of the mice with transplanted tumours was 22-24 days.

#### *Transplanted mouse cervical cancer model CUC-5 (epithelial tumour)*



Primary tumours were induced in female mice using methylcholantrene. A few primary tumours were then auto-transplanted into the uterine cervix. The life expectancy of mice with transplanted tumours was 43 days

#### *CBA Mice*

CBA female mice weighed 21.4 $\pm$ 1.2g, and were 2-3 months old. The tumours were introduced as a 0.5ml of tumour suspension (1g tumour per 10 ml of Igla medium) and ten mice used in each treatment group.

#### *Peptide preparation and administration*

Lyophilised peptide were dissolved in sterile physiological saline immediately before use in three concentrations. 0.5ml of solution was injected twice weekly into the peritoneum of the mice for the duration of the experiment. Three different concentrations of peptide were used leading to three final doses per injection per mouse of 10, 1.0 and 0.1 micrograms.

#### *Results*

The average percent decrease of tumour volume relative to the control group was recorded at three time points. The longevity of the mice in the treatment groups and control groups was compared and the histology of the tumours between control and treatment groups was compared

#### *Transplanted mouse sarcoma model SM-322*

Peptide	Concentration	Percent decrease in tumour volume relative to control			Days of life expectancy
	Microg/mouse	Day 8	Day 12	Day 15	
Rupe312	10	63	54	27	12-27
	1	67	32	38	12-19
	0.1	61	34	59	12-22
Rupe414	10	65	43	43	13-20
	1	68	28	43	12-20
	0.1	68	39	22	12-19
		Volume Day 8	Volume Day 12	Volume Day 15	
Control		1.49	11.2	17.7	12-16

#### *Transplanted mouse cervical cancer model CUC-5*

Peptide	Concentration	Percent decrease in tumour volume relative to control			Days of life expectancy
	Microg/mouse	Day 5	Day 12	Day 19	
Rupe312	10	50	25	49	48-76
	1	50	26	49	47-76
	0.1	50	18	45	42-76
Rupe414	10	50	16	48	36-76
	1	50	16	43	36-76
	0.1	50	18	57	47-76
		Volume Day 5	Volume Day 12	Volume Day 19	
Control		0.06	0.68	1.56	47-76

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13:KRRETVEREKE)

#### *Conclusion*

Hepreceptor peptides Rupe312 and Rupe414 reduce the growth rate of fast growing transplanted sarcomas leading to a slight (3-18%) increase in life expectancy.

Hepreceptor peptides Rupe312 and Rupe414 reduce the growth rate of slower growing transplanted cervical cancer but no significant increase (or decrease) in life expectancy was detected.

Hepreceptor peptides Rupe312 and Rupe414 induced a non bleeding ulcerous destruction of the centre of tumours in both models which consistently lead to smaller tumour volumes.

### EXAMPLE 3

**HEP1, a Hepreceptor peptide, administered orally (10mg per day either for thirty days or ninety days) to 21 HIV infected patients led to clinical improvement. The success of this study demonstrates generally the utility and reduction to practice of peptides derived from the Hepreceptor.**

This study was performed with pharmaceutical grade HEP1, a peptide which has an identical sequence to part of Domain A of the Hepreceptor. HIV-infected volunteers were recruited for the study at the Institute of Immunology, Moscow under the guidance of Professor Ravshan Ataullakhanov. The pharmaceutical grade HEP1 passed an extensive range of animal (rat and rabbit) toxicology and pre-clinical testing before the trial commenced, which demonstrated the safety of the compound. (Preliminary evaluation of toxicity- negative, Effect of 1000x therapeutic dose-negative, Local Irritation- negative, Influence on CNS and HVS-negative, Sub-acute toxicity-negative, Mutagenic effects-negative, Chronic toxicity-negative, Embryotoxicity- negative)

#### Study plan

Patients were orally administered a solution of 10mg of HEP1 in 2ml sterile distilled water once a day in the morning before breakfast ( the solution was prepared and stored in separate 10mg lots at  $-20^{\circ}\text{C}$ ). All patients were administered a coded placebo solution of distilled water for thirty days before treatment. A first group of 11 patients were administered HEP1 for 90 days and a second group of 10 patients were administered HEP1 for 30 days fifteen months later, after the data from the first group of patients had been analysed. During the treatment period the patients were requested to attend the clinic once a week, undergo a medical examination and give a blood sample for analysis. The patients were also requested to co-operate with post treatment monitoring and attend the clinic once a month for six months for further medical examinations and donations of blood samples. 21 out of 21 patients co-operated with monitoring during the treatment period and 14 out of 21 patients agreed to post treatment monitoring. The patients were not receiving any other anti-retroviral therapy during or one month before HEP1 treatment.

### Patients

Patients were recruited from various clinics around Moscow and gave informed written consent to participate in the trial. They were identified as HIV infected by a positive ELISA assay, had depressed T cell counts and experiencing some clinical manifestation of HIV related illness. The patients were subsequently shown to have a range of CD4 cells per microlitre between 17 and 801 and a range of serum HIV RNA (Roche Labs Amplicor quantitative PCR assay) from undetectable to 230,000 copies per ml.

### Patient characteristics at start of trial

ID Code	Sex	Age	Est Period of infection	CD4 cells / microL	HIV RNA Copies / ml	opportunistic infections	other
P1	male	45	8	219	500	severe	
P2	male	33	2	192	10000	severe	
P3	female	45	6	481	2000	severe	
P4	female	16	8	237	43000	severe	
P5	male	27	2	123	<400	moderate	very sick
P6	female	23	1	357	10000	moderate	Herpes z
P7	male	23	8	139	94000	very low	Herpes z
P8	female	38	10	320	22000	very low	Ovarian cyst
P9	male	43	3	17	10000	severe	
P10	male	19	2	155	21000	severe	On opiates
P11	male	35	3	188	<400	severe	Active TB
P12	male	32	8	175	13000	severe	
P17	male	25	1	478	11000	severe	
P21	female	37	10	98	11000	severe	
P63	male	35	1	651	4000	severe	
P67	female	31	7	124	230000	severe	Very sick
P68	female	51	2	597	<400	severe	Very sick
P69	male	34	1	192	8000	severe	Active TB
P72	male	33	1	534	25000	severe	Active TB
P73	male	30	1	801	<400	severe	Active TB
P76	female	38	7	72	9000	severe	

### General observations

The patients reported no adverse reactions to HEP1, and 17 patients reported they felt generally better and gained at between 1.5Kg and 4.5Kg in weight while on HEP1 therapy (one patient also felt better during the placebo period).

**Adverse reactions**

No adverse reactions were detected. Clinical assessments including ultrasound examinations and an extensive series of biochemical, haematological, immunological blood tests and urine tests were performed.

**Opportunistic infections**

Opportunistic infections were detected by microbiological analysis and patients treated by HEP1 were either stable with no new infections or infections declined. For example, before treatment 38% of the patients had severe *Candida Albicans* infection of the pharynx, after treatment only 9% were severely infected. Before treatment, 52% of the patients had severe *S. viridans* infection of the pharynx, after treatment only 33% were severely infected. Before treatment, 33 % of the patients had *S. aureus* infection of the pharynx, after treatment only 19 % were infected.

**CD4 T lymphocytes**

The group eleven patients were treated for three months with HEP1 experienced an average gain of T cell numbers of 9% by the end of treatment and 32% on average over the following six months after treatment. The group ten patients who were treated for one month with HEP1 experienced an average gain of T cell numbers of 3% by the end of treatment and 20% on average over the following six months after treatment. The continued improvement suggests that some positive immunological change had been induced in the patients.

**HIV infectivity assay by TCID**

Viral load was measured by mixing HIV infected Peripheral Blood Mononuclear Cells (PBMC) from the patient with uninfected donor PBMC in a ratio between sample cells to culture cells of 1/16 and culturing at 37°C for fourteen days. Culture viral load was measured by an Innogenetics HIVp24 assay and the results were recorded in picograms of HIVp24 antigen per ml.

**Three month treatment**

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Infectivity in pg/ml p24max at start of trial	1039	315	na	386	515	203	1113	369	1074	na	480
re-based to 100 at start	100	100	0	100	100	100	100	100	100	0	100
Phase 0 average						130					
Phase 1 average	7	32		0	28	0	47	0	39		
Phase 2 average	21	218		198	129	251	70	217	40		835
Phase 3 average	0	61		0	85	0	48		24		

**One month treatment**

Patient	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
Infectivity in pg/ml p24max at start of trial	465	0.3	31	2.9	6642	6.5	3.2	2	1	4.5
re-based to 100 at start	100	100	100	100	100	100	100	100	100	100
Phase 0 average	288							251		
Phase 1 average	24	0			5		29	0		73
Phase 2 average	1815	209	6924	659	334	993	28919	115	236	141
Phase 3 average	5	39	54	51	51		15	57		102

In both groups of patients (P1-P11 and P12-P76), a general pattern of infectivity was observed in the TCID assay for detecting infectious virus particles. At the beginning of HEP1 therapy, (Phase 0- Phase 1), the load of infectious HIV virus declined sharply to low levels within the first three weeks of treatment and in 9 out of 21

patients dropped to zero for at least one week. Three patients experienced an increase in infectivity during the first week of treatment before infectivity dropped to zero in the second week. In the second phase which followed, (Phase 2), in the majority of patients HIV infectivity rose between 2X and 600X between four and eight weeks after the start of treatment. The patients reported no worsening of their condition during this period. Phase 3 followed where infectivity declined to below pre-treatment levels.

In Phase 1, the average maximum decline of virus levels below the pre-treatment baseline was minus 80%. In Phase 2, the average maximum increase of virus in Phase Two was 22 times. In Phase 3, the average maximum decline of virus below the pre-treatment baseline was minus 64%. During Phase 3, viral infectivity declined to zero in three patients. Six months after the end of treatment with HEP1, viral infectivity generally returned to pre-treatment levels.

I interpret these results as showing that the immune system was activated by HEP1 to fight HIV in Phase One. The increased level of activation in the immune system stimulated activation of a reservoir of cells latently infected with HIV leading to the increase in infectious virus in Phase 2. Finally in Phase 3, the activated immune system successfully destroyed the newly activated virus reservoir. The group of ten patients treated for only one month with HEP1 showed that the progression through Phase 2 and Phase 3 did not depend on the presence of HEP1.

#### HIV viral load by quantitative plasma HIV RNA PCR

Analysis of viral load was performed by the Roche labs PCR assay: Amplicor HIV-1 Monitor.

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
HIV plasmaRNA in 1000s copies /ml	0.5	10	2	43	<0.4	10	94	22	10	21	<0.4
re-based to 100 at start	100	100	100	100	0	100	100	100	100	100	0
Phase 0 average						108	143		101		
Phase 1 average			38	39	28	37	68	78	90	45	
Phase 2 average	476	197	103	64	28	223	112	110	1535	92	
Phase 3 average		85	66	77	0	64	92	52		39	

Patient	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
HIV plasmaRNA in 1000s copies /ml	13	11	11	4	230	<0.4	8	25	<0.4	9
re-based to 100 at start	100	100	100	100	100	0	100	100	100	100
Phase 0 average										
Phase 1 average		0			78					55
Phase 2 average	219	177	273	206	91		142	191		139
Phase 3 average	135	86		98	63		138	72		72

In the majority of patients of both the one month and three month treatment, similar phases of viral suppression (Phase 1) followed by temporary viral activation (Phase 2) follow by suppression (Phase 3) were observed which was similar to the TCID data. Phase I lasted between one and four weeks and viral load declined on average by minus 47%. Phase 2 lasted between eight and forty weeks (a more sustained period than seen with the TCID assay) and viral RNA in the plasma increased on average by 3X. This was followed by Phase 3 in which viral RNA in the plasma fell below pre-treatment levels by an average reduction of minus 19%.

### Cell populations and expression of cell surface markers by cytometry

Cells surface markers on peripheral blood mononuclear cells of the group of ten patients (P12-P76) treated for one month with HEP1 were analysed in detail using microscopy, fluorescent labelled antibodies, flow cytometry and related procedures. HEP1 treatment had the following effects on each cell populations averaged for the 10 patient group:

a) Lymphocytes

an average increase of 7 % in absolute number of lymphocytes during treatment and an increase of 25% for the five months following treatment.

b) Leucocytes

an average increase of 10% during treatment and an increase of approximately 20% for the five months following treatment.

d) Natural Killer Cells

An average increase of 10% during treatment and an increase of approximately 30% for the five months following treatment

e) B cells

An average increase of 5% during treatment and an increase of approximately 80% for the five months following treatment

f) CD3 expressing cells

An average increase of 15% during treatment and an increase of approximately 30% for the five months following treatment

g) CD8 expressing cells

An average increase of 15% during treatment and an increase of approximately 20% for the five months following treatment

h) CD44-CD4 expressing cells

An average increase of 25% during treatment and an increase of approximately 60% for the five months following treatment

h) CD44-CD8 expressing cells

An average increase of 12% during treatment and an increase of approximately 30% for the five months following treatment

i) HLA ClassI expression level on CD4 cells

An average increase of 10% during treatment and an increase of approximately 70% for the five months following treatment

j) HLA ClassI expression level on CD8 cells

An average increase of 10% during treatment and an increase of approximately 70% for the five months following treatment.

k) CD25-CD8 expressing cells

An average increase of 5% during treatment from low levels and an increase of approximately 100% for the five months following treatment. CD25-CD4 cells did not show significant variation.

l) CD38-CD4 expressing cells

An average increase of 2% during treatment from low levels and a decrease of approximately 15% for the five months following treatment.

m) CD38-CD8 expressing cells

An average increase of 2% during treatment from low levels and a decrease of approximately 25% for the five months following treatment. (the significant decrease in CD38 is discussed in the next section)

Other markers such as CD28, HLA-DR, CD45RO, CD45RA, CD57, CD62L, showed no significant changes or consistent patterns between patients.

#### **CD38-CD8 cells: prognostic indicator for progression to AIDS**

It is well recognised that the increasing size of the population of cells expressing CD38-CD8 correlates with the development of AIDS in HIV infected patients.

M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)

"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* 90 376-382

Briefly, the CD38-CD8 cells as a percent of the total CD8 population correlate with HIV disease progression, an observation which has been verified in a number of more recent publications. In healthy people, the percentage of the CD8 cells which also express CD38 is between 30-50%, in asymptomatic HIV infected patients is between 50-65%, in HIV infected patients with ARC is between 65% and 80% and in AIDS patients between 80% and 98%. In the follow up of the study of ten patients who took 10 mg HEP1 orally for one month, 5 patients provided blood samples for analysis. In all of these patients it was clear that the population of CD38-CD8 cells as a percent of the total CD8 population declined toward values indicating lower risk of HIV disease and improving health.

CD38-CD8 cells as a % CD8 cells	Baseline	Month 1 treatment	Month 2	Month 3	Month 4	Month 5	Month 6
Patient 12	92	94		79	84	73	72
Patient 17	66	71		69	50	55	57
Patient 21	83	86				66	
Patient 69	90	94		86	79	71	
Patient 76	78	84		77	64	54	50

#### **Anti-HIV antibodies in plasma**

At the end of one month treatment with HEP1 of 10 patients (P12-P76), 5 patients showed significantly higher antibody titres against various HIV antigens (titres re-based to 100 before treatment).

	Anti gp120	Anti gp41	Anti-p31	Anti p24	Anti p17
Patient 17	216	93	157	97	101
Patient 21	127	110	71	103	110
Patient 63	110	131	387	146	154
Patient 69	160	274	813	507	116
Patient 72	93	111	147	245	143

**Antibody responses to opportunistic infections**

HEP1 treatment stimulated antibody responses to opportunistic infections (antibody titre re-based to 100 before treatment)

Max titre during treatment	P12	P17	P21	P63	P67	P68	P69	P72	P73	P76
Aspergillus IgG	122	97	132	116	89	114	119	190	142	111
Candida IgG	127	100	126	170	447	51	293	214	120	129
CMV IgG	290	81	227	186	118	91	105	112	152	88
CMV IgM	120	141	142	215	159	92	102	148	120	142
HSV1 IgM	113	129	158	134	99	89	116	101	107	106
HSV2 IgM	107	129	109	108	156	158	146	111	300	186
Toxoplasma IgG	105	107	98	108	125	101	104	95	105	98

The average increase in antibody titre was plus 23% for Aspergillus IgG, plus 78% for Candida IgG, plus 50% for CMV IgG, plus 38% for CMV IgM, plus 15% for HSV1 IgM, plus 51% for HSV2 IgM and plus 5% for Toxoplasma IgG.

**Conclusion**

The above data is consistent with the invention that a peptide with a sequence identical to part of the Hepreceptor leads to immune activation and clinical benefits in a human clinical trial of HIV patients.



**EXAMPLE 4**

**Severe acute and chronic Candida infection in women can be cured by the immune response resulting from Heptareceptor stimulation.**

**Clinical Study: recurrent moderate candida infection**

Female (age 27) with an untreated fresh out-break of Candida infection volunteered for the study. She reported recurrent moderate vaginal Candida infection (six episodes in previous twelve months) which had been previously treated with intra-vaginal application of 1% clotrimazole. She self-administered 5ml of a 1mg/ml solution of HEP1 intra-vaginally with a 5ml syringe on two consecutive days. After three days all clinical symptoms of Candida infection had disappeared and she reported no further recurrences of Candida infection in the 12 month follow-up.

**Clinical Study: severe persistent candida infection**

Three female patients attending the Nearmedic Plus STD clinical in Moscow volunteered for the study who were suffering from severe candida infection of the vagina after they had been treated with antibiotics for various genitourinary infections. The patients were treated with 5ml of a 2mg/ml solution of HEP1 for three consecutive days (no other antifungal treatment was used). Comparison of microbiological analysis (cultivation of urethral, cervical canal and vaginal swabs) and clinical analysis before treatment and three weeks after treatment demonstrated either significant improvement or elimination of the infection.

Patient	Age	Period	Clinical analysis	Urethra swab	Cervical swab	Vaginal swab
LLA	34	Before	Severe infection	Intense growth	Low growth	Low growth
KEM	28	Before	Severe infection	Intense growth	Intense growth	Intense growth
ALN	35	Before	Severe infection	Intense growth	Intense growth	Intense growth
LLA	34	After	No symptoms	Absent	Absent	Absent
KEM	28	After	mild symptoms	Low growth	few	Absent
ALN	35	After	No symptoms	Absent	Absent	Absent

**EXAMPLE 5**

**Rupe312, Rupe414, Rupe111 and Rupe411 induce a strong macrophage activation response in mice.**

A number of peptides derived from of the Hephreceptor Domain A including HEP1 were studied in mice.

**Induction of activated macrophages**

Groups of three mice (CDAxC57Bl)F1 weighing 22-24g were injected abdominally with each peptide solution of 1.0 microgram of peptide dissolved in 0.5ml of physiological saline. After 24 hours the animals were killed using neck vertebrae dislocation and 5 ml of Hanks solution was injected into the abdomen. The abdomen was massaged for 30 seconds and then the peritoneal liquid was collected. The collected liquid was filtered using a nylon filter into siliconised tubes containing 1.5mg/ml EDTA.

The number of nucleus containing cells in 1microgram of filtrate were then assessed under microscopic examination using a Nihon hemocytometer. The cells were pelleted by centrifugation for 5 minutes at 800g, the pellet was resuspended in fetal calf serum, the cell suspension was dropped on to a glass microscope slide and dried then fixed in methanol and stained with Romanovski's colouring agent. Morphological analysis of the cells of the peritoneal exsudate were performed using an Opton optical microscope at 1600 magnifications. The number of lymphocytes, resting macrophages, activated macrophages, granulocytes and other cell types were assessed. The result was that the peptides all increased the number of activated macrophages but Rupe111, Rupe312, Rupe411 and Rupe414 were significantly more active than HEP1.

1.0 microgram peptide /mouse	saline	HEP1	Rupe111	Rupe312	Rupe411	Rupe414
Activated macrophages as a percent of total number of cells	1.9	2.2	4.3	13.9	5.5	8.5

HEP1	SEQ ID 28	TEKKRRETVEREKE
Rupe111	SEQ ID 5:	TEKKRRETVER
Rupe312	SEQ ID 8:	KKRRETVERE
Rupe411	SEQ ID 11:	KRRETVER
Rupe414	SEQ ID 13:	KRRETVEREKE

**EXAMPLE 6**

**The in vitro activation of human peripheral blood mononuclear cells by Hephreceptor peptides demonstrated by measuring the incorporation of radioactive tritiated thymidine into the DNA of growing cells.**

Peripheral Blood Mononuclear Cells (PBMC) were separated from the peripheral blood of a healthy donor using the standard method of fractionation in a ficoll gradient. The PBMC were suspended in culture medium containing RPMI1640 medium plus 10% fetal calf serum, 1mM L-glutamin and antibiotics (BM). The cell suspension was placed in wells of a 96 hole microwell plate for cell cultivation, (100 microlitres of suspension containing 100,000 cells per well). Then 100 microlitres of BM was added containing peptide ( final concentration 0.001-10 microgram/ml). The negative control well contained BM but no peptide. The plate was incubated at 37°C for three days then radioactive <sup>3</sup>H thymidine was added to a final concentration of 1 microcurie per ml. The incorporation of <sup>3</sup>H thymidine into the DNA of the cells was measured using a betacounter using standard procedures. The experiment was repeated twice and the results expressed as an average of the two experiments in radioactive counts per minute. The result showed that all the peptides activate mononuclear cell proliferation but that Rupe312 and Rupe414 were significantly more active than HEP1 with peak activity around 3 nanograms/ml.

Peptide microg/ml	Control	HEP1	Rupe19	Rupe312	Rupe414	Rupe411	Rupe111	Rupe614
0.0001	370	410	426	563	493	385	483	464
0.0003	370	500	602	742	580	510	483	503
0.001	370	989	718	976	684	702	710	550
<b>0.003</b>	<b>370</b>	<b>700</b>	<b>756</b>	<b>3222</b>	<b>2087</b>	<b>598</b>	<b>665</b>	<b>752</b>
0.01	370	628	545	656	650	532	537	607
0.03	370	517	586	539	596	500	642	538
0.1	370	456	537	533	485	499	633	596
0.3	370	399	563	611	492	486	668	635
1	370	400	509	472	449	468	529	600
3	370	412	502	455	437	420	486	499
10	370	501	517	405	394	390	470	412

HEP1	SEQ ID 28	TEKKRRETVEREKE
Rupe19	SEQ ID 4:	TEKKRRETV
Rupe312	SEQ ID 8:	KKRRETVERE
Rupe414	SEQ ID 13:	KRRETVEREKE,
Rupe411	SEQ ID 11:	KRRETVER
Rupe111	SEQ ID 5:	TEKKRRETVER
Rupe614	SEQ ID 18:	RETVEREKE

**EXAMPLE 7****Effect of Hепreceptor peptides on expression of MHC Class I on various immunological cells**

The incubation of Hепreceptor derived peptides (0.003 micrograms per ml) with human White Blood Cells (WBC) for 24 hours at 37°C, resulted in a fall in the intensity of HLA expression on the cell surface of all WBC (due to cell activation and receptor internalisation). Rupe 312 was more active than Rupe 414 which was more active than HEP1.

Data rebased to 100 for the control value in the absence of peptides

Density of cell surface expression of HLA Class I					
	Monocytes / Macrophages	CD8 lymphocytes	CD4 lymphocytes	B and NK cells	Granulocytes
Control	100	100	100	100	100
HEP1	83	83	80	84	89
Rupe414	83	76	77	77	85
Rupe312	71	72	70	73	81

A cell specific effect of this activation was an increase in the population of monocytes expressing MHC Class I and a decrease in the population of CD8 lymphocytes expressing MHC Class I.

Data rebased to 100 for the control value in the absence of peptides

Percentage of cell population expressing HLA Class I					
	Monocytes / Macrophages	CD8 lymphocytes	CD4 lymphocytes	B and NK cells	Granulocytes
Control	100	100	100	100	100
HEP1	108	91	105	102	100
Rupe414	109	89	105	101	98
Rupe312	119	85	104	105	98

(Rupe312 SEQ ID 8:KKRRETVERE and Rupe414 SEQ ID 13:KRRETVEREKE)

### EXAMPLE 8

#### Hepreceptor peptides suppresses IL-8 production in WBC

The suppressive effect of increasing concentrations of Rupe312, a Hepreceptor peptide, on the expression of IL-8 by human WBC after a 48 hour incubation at 37°C was detected. IL-8 is a chemotactic factor that is produced in response to inflammatory stimulus which attracts and activates T cells, neutrophils, basophils, granulocytes but not monocyte/macrophages. The inhibition of IL-8 may play a role in the selective activity of Rupe 312 in activating monocytes macrophages. The measurement of IL-8 provides an assay for determining the activity of various Hepreceptor derived peptides.

Rupe312 concentration in micrograms/ml	IL-8 concentration in culture in picrograms/ml
0	18900
0.001	13900
0.003	10700
0.01	8984
0.03	7869
0.1	6426

(IL-8 EIA assay manufactured by Innogenetics, Belgium)  
(Rupe312 SEQ ID 8:KKRRETVERE)

### EXAMPLE 9

#### Very low dose Hepreceptor peptides (1-100 nanograms/mouse) protect mice from acute infection by Salmonella typhimurium

Laboratory mice (CBAXC57Bl F1 hybrids) were split into groups of five which either received 0.5ml saline or 0.5ml saline plus various concentrations of different Hepreceptor peptides (1,10 or 100 nanograms). 24 hours later the mice were acutely infected with Salmonella typhimurium (10,000 or 100,000 bacteria injected intraperitoneally per mouse). The percent of each group of mice surviving after 20 days was recorded (lethally infected control animals were dead within three days of infection).

Percent of mouse group surviving after 20 days infection

Peptide code	1000s bacteria per mouse	Control	Hepreceptor peptides in nanograms per mouse				Peptide sequence
		0	1	10	100		
HEP1	10	0%	20%	20%	40%		TEKKRRETVEREKE
	100	0%	0%	0%	0%		
Rupe15	10	0%	20%	40%	40%		TEKKR
	100	0%	20%	20%	0%		
Rupe1024	10	0%	40%	40%	40%		EREKEQMMREKEEL
	100	0%	0%	20%	40%		

(Rupe15 SEQ ID 3: TEKKR and Rupe1024 SEQ ID 17: EREKEQMMREKEEL and HEP1 SEQ ID 28: TEKKRRETVEREKE)

**Conclusion**

Hepreceptor peptides protect animals from lethal bacterial infection.

**EXAMPLE 10**

**Very low dose Hepreceptor peptides (1-1000 nanograms/mouse) enhance survival time in mouse lethal herpes virus infection model**

Laboratory white B/P mice (five per group) were injected intraperitoneally with Hepreceptor peptide (1-1000 nanograms per mouse,) 48 hours and 24 hours before a lethal injection of herpes virus (VPG-1 strain L2) at a titre of 3.5 LD50 in 0.2ml medium.

The average survival time in days was recorded per group of mice.

Peptide code	Control	Hepreceptor peptide in nanograms per mouse					Peptide sequence
		0	1	10	100	1000	
HEP1	10	18	19	27	28		TEKKRRETVEREKE

(HEP1 SEQ ID 28: TEKKRRETVEREKE)

**Conclusion**

Hepreceptor peptides significantly enhance survival time from lethal viral infection

**PRIORITY DOCUMENTS**

UK patent application GB9921881.0, Holms R., 17<sup>th</sup> September 1999

**PATENT DOCUMENTS**

United States Patent 5,773,573

Rupert Holms 30<sup>th</sup> June 1998

**OTHER PUBLICATIONS**

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Anthony Bretscher, David Reczek and Mark Berryman (1997)

"Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures" *Journal of Cell Science* **110**: 3011-3018

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M Levancher, F Hulstaert, S. Tallet, S Ullery, J J Pocidalo, B A Bach (1992)

"The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value" *Clinical Experimental Immunology* **90** 376-382

Stephan Oehen and Karin Brduscha-Riem (1998)

"Differentiation of Naïve CTL to Effector and Memory CTL: Correlation of Effector Function with Phenotype and Cell Division" *The Journal of Immunology* **161** 5338-5346

## CLAIMS

1. a charged molecule that binds to the Heparin receptor
2. a peptide with a sequence identical to part of the Heparin receptor
3. a peptide of claim 2, between five and thirteen amino acids in length which has a sequence identical to part of the Heparin receptor
4. a peptide comprising of the amino acid sequence;  
AREEKHQKQLERQQLETEKKRRRETVEREKEQM
5. a peptide comprising of the amino acid sequence;  
MREKEELMLRLQDY<sub>(p)</sub>EEKTKKAERELSEQIQRALQ
6. a peptide comprising of the amino acid sequence;  
TEKKR
7. a peptide comprising of the amino acid sequence;  
TEKKRRRET
8. a peptide comprising of the amino acid sequence;  
TEKKRRRETVER
9. a peptide comprising of the amino acid sequence;  
KKRRE
10. a peptide comprising of the amino acid sequence;  
KKRRETVE
11. a peptide comprising of the amino acid sequence;  
KKRRETVERE
12. a peptide comprising of the amino acid sequence;  
KKRRETVEREK
13. a peptide comprising of the amino acid sequence;  
KKRRETVEREKE
14. a peptide comprising of the amino acid sequence;  
KRRETVER
15. a peptide comprising of the amino acid sequence;  
KRRETVEREK
16. a peptide comprising of the amino acid sequence;  
KRRETVEREKE
17. a peptide comprising of the amino acid sequence;  
RRET
18. a peptide comprising of the amino acid sequence;  
RETVEREKE
19. a peptide comprising of the amino acid sequence;  
EREKE
20. a peptide comprising of the amino acid sequence;  
EREKEQMMREKEEL
21. a peptide comprising of the amino acid sequence;  
KEELM
22. a peptide comprising of the amino acid sequence;  
KEELMLRLQDYEE
23. a peptide comprising of the amino acid sequence;  
KEELMLRLQDY<sub>p</sub>EE
24. a peptide comprising of the amino acid sequence;  
EELMLRLQDYEE
25. a peptide comprising of the amino acid sequence;  
EELMLRLQDY<sub>p</sub>EE



- 26. a peptide comprising of the amino acid sequence;  
ELMLRLQDYEE
- 27. a peptide comprising of the amino acid sequence;  
ELMLRLQDYpEE
- 28. a peptide comprising of the amino acid sequence;  
MLRLQ
- 29. a peptide comprising of the amino acid sequence;  
QDYEE
- 30. a peptide comprising of the amino acid sequence;  
QDYpEE

## Primary/secondary structure of the Heptareceptor

~ = continuity of aminoacid sequence  
p = phosphate group on tyrosine

[illegible] $\frac{1}{2}$ 

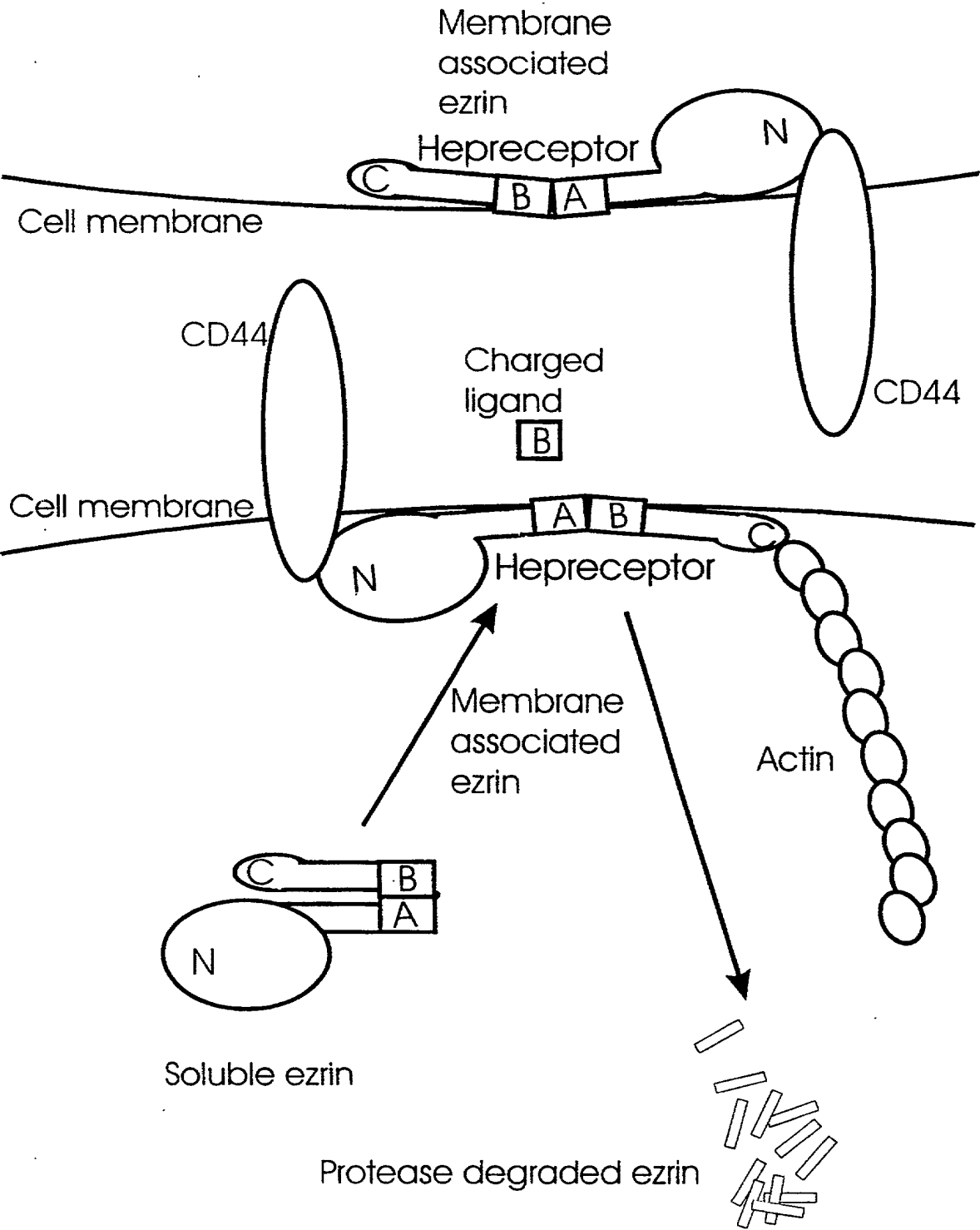
Hepreceptor1  
inverted sequence  
Side chain charge  
aminoacid  
seq number

373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

domain B  
domain A  
Hinge  
D

[illegible]

Cellular locations and complexes of ezrin



## DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of subject matter which is claimed and for which a patent is sought on an invention entitled  
**REGULATORY/UNFOLDING PEPTIDES OF EZRIN**

the specification of which ☐ is attached hereto or

☐ was filed on 15 SEP 2000 as United States Application Number or PCT International Application Number PCT/GB00/03566 and was amended on (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for a patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application Number(s)	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
9921881.0	GB	17 SEPT 1999	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:  
 David R. Saliwanchik, Reg. 31,794; Jeff Lloyd, Reg. 35,589; Doran R. Pace, Reg. 38,261; Christine Q. McLeod, Reg. 36,213; Jay M. Sanders, Reg. 39,355; James S. Parker, Reg. 40,119 and Jean E. Kyle, Reg. 36,987; Frank C. Eisenschenk, Reg. 45,332; Seth M. Blum. Reg. 45,489

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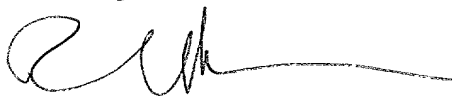
9

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C 1001 and that such willful false statements may jeopardise the validity of the application or any patent issued thereon.

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Date of signature

1/5/01

## SEQUENCES LISTING

### GENERAL INFORMATION

NUMBER OF SEQUENCES: 28

### INFORMATION FOR SEQ ID 1

#### SEQUENCE CHARACTERISTICS:

LENGTH: 32 amino acids  
 TYPE: amino acid  
 STRANDEDNESS: single  
 TOPOLOGY: linear  
 MOLECULAR TYPE: peptide

#### SEQUENCE DESCRIPTION

SEQ ID 1

Hepreceptor Domain A

A R E E K H Q K Q L E R Q Q L E T E K K R R E T V E R E K E Q M  
 1            5            10            15            20            25            30

### INFORMATION FOR SEQ ID 2

#### SEQUENCE CHARACTERISTICS:

LENGTH: 34 amino acids  
 TYPE: amino acid  
 STRANDEDNESS: single  
 TOPOLOGY: linear  
 MOLECULAR TYPE: peptide

#### SEQUENCE DESCRIPTION

SEQ ID 2

Hepreceptor Domain B

M R E K E E L M L R L Q D Y<sub>(p)</sub> E E K T K K A E R E L S E Q I Q R A L Q  
 1            5            10            15            20            25            30

### INFORMATION FOR SEQ ID 3

#### SEQUENCE CHARACTERISTICS:

LENGTH: 5 amino acids  
 TYPE: amino acid  
 STRANDEDNESS: single  
 TOPOLOGY: linear  
 MOLECULAR TYPE: peptide

#### SEQUENCE DESCRIPTION

SEQ ID 3

Rupe15:            T E K K R  
                      1        5

**INFORMATION FOR SEQ ID 4****SEQUENCE CHARACTERISTICS:**

LENGTH: 9 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 4

Rupe19: TEKKRRETV  
1 5

**INFORMATION FOR SEQ ID 5****SEQUENCE CHARACTERISTICS:**

LENGTH: 11 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 5

Rupe111: TEKKRRETVER  
1 5 10

**INFORMATION FOR SEQ ID 6****SEQUENCE CHARACTERISTICS:**

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 6

Rupe37: KKRRE  
1 5

**INFORMATION FOR SEQ ID 7****SEQUENCE CHARACTERISTICS:**

LENGTH: 8 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 7

Rupe310: KKRRETVE  
1 5

**INFORMATION FOR SEQ ID 8****SEQUENCE CHARACTERISTICS:**

LENGTH: 10 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 8

Rupe312: KKRRETVERE  
1 5 10

**INFORMATION FOR SEQ ID 9****SEQUENCE CHARACTERISTICS:**

LENGTH: 11 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 9

Rupe313: KKRRETVEREK  
1 5 10

**INFORMATION FOR SEQ ID 10****SEQUENCE CHARACTERISTICS:**

LENGTH: 12 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 10

Rupe314: KKRRETVEREKE  
1 5 10

**INFORMATION FOR SEQ ID 11****SEQUENCE CHARACTERISTICS:**

LENGTH: 8 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 11

Rupe411: KRRETVER  
1 5



**INFORMATION FOR SEQ ID 12****SEQUENCE CHARACTERISTICS:**

LENGTH: 10 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 12

Rupe413: KRRETVEREK  
1 5 10

**INFORMATION FOR SEQ ID 13****SEQUENCE CHARACTERISTICS:**

LENGTH: 11 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 13

Rupe414: KRRETVEREKE  
1 5 10

**INFORMATION FOR SEQ ID 14****SEQUENCE CHARACTERISTICS:**

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 14

Rupe59: RRETV  
1 5

**INFORMATION FOR SEQ ID 15****SEQUENCE CHARACTERISTICS:**

LENGTH: 9 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 15

Rupe614: RETVEREKE  
1 5

**INFORMATION FOR SEQ ID 16****SEQUENCE CHARACTERISTICS:**

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 16

Rupe1014: EREKE  
1 5

**INFORMATION FOR SEQ ID 17****SEQUENCE CHARACTERISTICS:**

LENGTH: 14 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 17

Rupe1024 EREKEQMMREKEEL  
1 5 10

**INFORMATION FOR SEQ ID 18****SEQUENCE CHARACTERISTICS:**

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 18

Rupe2024: KEELM  
1 5

**INFORMATION FOR SEQ ID 19****SEQUENCE CHARACTERISTICS:**

LENGTH: 13 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 19

Rupe2032: KEELMLRLQDYEE  
1 5 10

**INFORMATION FOR SEQ ID 20****SEQUENCE CHARACTERISTICS:**

LENGTH: 13 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 20

Rupe2032p: KEELMLRLQDYpEE  
1 5 10

**INFORMATION FOR SEQ ID 21****SEQUENCE CHARACTERISTICS:**

LENGTH: 12 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 21

Rupe2132: EELMLRLQDYEE  
1 5 10

**INFORMATION FOR SEQ ID 22****SEQUENCE CHARACTERISTICS:**

LENGTH: 12 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 22

Rupe2132p: EELMLRLQDYpEE  
1 5 10

**INFORMATION FOR SEQ ID 23****SEQUENCE CHARACTERISTICS:**

LENGTH: 11 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

**SEQUENCE DESCRIPTION**

SEQ ID 23

Rupe2232: ELMLRLQDYEE  
1 5 10

**INFORMATION FOR SEQ ID 24**

## SEQUENCE CHARACTERISTICS:

LENGTH: 11 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

## SEQUENCE DESCRIPTION

SEQ ID 24

Rupe2232p: ELMLRLQDYpEE  
1 5 10

**INFORMATION FOR SEQ ID 25**

## SEQUENCE CHARACTERISTICS:

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

## SEQUENCE DESCRIPTION

SEQ ID 25

Rupe2428: MLRLQ  
1 5

**INFORMATION FOR SEQ ID 26**

## SEQUENCE CHARACTERISTICS:

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

## SEQUENCE DESCRIPTION

SEQ ID 26

Rupe2832: QDYEE  
1 5

**INFORMATION FOR SEQ ID 27**

## SEQUENCE CHARACTERISTICS:

LENGTH: 5 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

## SEQUENCE DESCRIPTION

SEQ ID 27

Rupe2832p: QDYpEE  
1 5

## INFORMATION FOR SEQ ID 28

## SEQUENCE CHARACTERISTICS:

LENGTH: 14 amino acids  
TYPE: amino acid  
STRANDEDNESS: single  
TOPOLOGY: linear  
MOLECULAR TYPE: peptide

## SEQUENCE DESCRIPTION

SEQ ID 28

HEP1: TEKKRRETVEREKE  
1 5 10